EXCEPTIONAL INHERITANCE OF A SEX-LINKED GENE IN THE MOUSE EXPLAINED ON THE BASIS THAT THE X/O SEX-CHROMOSOME CONSTITUTION IS FEMALE

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Until recent years, no sex-linked genes were known in the mouse. Thus, the tools for the genetic exploration of sex determining mechanisms were lacking. A sex-linked mutation, *scurfy*, discovered at this laboratory in 1949 has, on occasions, shown unexpected inheritance. In spite of the lethal effects of the mutation, it has been possible to carry out genetic analysis of the exceptional individuals (*scurfy* females) by means of ovarian transplantation. As a result, the array of possible explanations for the exceptional individuals could be narrowed down. Several alternative hypotheses, however, remained, with no decision possible between them. In the meantime, other sex-linked genes and adequate cytological techniques became available, and recent findings by Welshons and Russell[1] have shed much light on the sex-determining mechanism of the mouse. The present paper will describe our older work on the genetic analysis of the *scurfy* exceptions and show that the results are quite consistent with the proof, presented in the accompanying paper,[1] that X/O is female.

Material and Methods.—The mutation *scurfy*, *sf*, occurred spontaneously in our MR-stock, a stock which was then in the process of being made homozygous for 7 standard recessives, most of them coat colors. Details concerning the phenotypic expression, derivation, and transmission of *sf* will be published elsewhere. *Scurfy* is inherited as a sex-linked recessive gene. Hemizygous males are characterized by a number of features. The external characteristics are: scaliness, first of the tail and later of other parts of the body; "tight" skin; and late opening of the eyelids. The condition is first recognizable externally at about 11 days of age by a reddening of the genital papilla. About two-thirds of the affected males die before weaning age, most of the remaining ones shortly after weaning. Occasional males have lived several months, one as long as six months, but all of these are runty and none has been fertile. Thus, the stock must be maintained by mating X^{sf}/X^{+} females with X^{+}/Y males. Consequently, females with the *scurfy* phenotype were not expected to occur.

Early in 1950, the first exceptional, *scurfy*, female was observed in the stock and a total of 29 have been obtained to date. (Frequency will be discussed below.) These exceptional females were phenotypically similar to X^{sf}/Y males. Since they were presumably destined to die without reproducing, it was necessary to resort to ovarian transplantation in order to carry out genetic analysis, and ovaries from the first 13 cases that occurred were transplanted, mostly during the years 1950–1951. The fact that *scurfy* females do, indeed, die without reproducing was confirmed by the next 15 cases, which were not operated upon and died aged 16–45 days. Ovaries from the 29th *scurfy* female to be observed were transplanted in 1958.

It is, of course, desirable to have genetic markers such that offspring of transplanted ovaries can be distinguished from offspring of regenerated host ovaries.[2]

During the earlier part of the 1950–1951 period, this condition was only partly met. The gene *sf* was available only on a mixed background containing a number of recessive markers, mostly coat colors, and having an ancestry of one or more crosses to (101 × C3H)F_1. The hosts chosen for 10 of the 13 early operations were (101 × C3H)F_1 females, wild-type for all coat color genes, and these were mated to males homozygous for all of the recessive markers. Thus, any young non-wild-type with respect to the markers were definitely derived from the grafted ovary. On the other hand, not all wild-type young were necessarily derived from the regenerated host ovary. This should be kept in mind in the discussion of the results. It was, of course, possible that the donor-host combination in these operations would be incompatible. However, it was hoped that the choice of (101 × C3H)F_1 females as hosts had increased the probability of success of grafting. In case this hope did not materialize, a second approach was initiated. As soon as *scurfy* females first occurred, introduction of the *sf* gene into the 129-strain was started. This inbred strain, which maintains c^{ch} and c^a in forced heterozygosis, was developed by us several years ago for the purpose of providing an isogenic background containing markers which would permit unequivocal distinction between offspring from transplanted and regenerated ovaries.[2] It was possible to use this more suitable material for the latter 3 of the 13 early ovarian transplant operations involving *scurfy* females and for the fourteenth operation, which was carried out in 1958. In these operations, the hosts chosen were 129-strain females of genotype c^ac^a who received ovaries from $c^{ch}c^{ch}$ *scurfy* females and were then mated to $c^{ch}c^{ch}$ males, thus producing $c^{ch}c^a$ young from regenerated host ovarian tissue and $c^{ch}c^{ch}$ young from successful graft ovaries.

The transplant operations were carried out when the *scurfy* females were between 15 and 31 days old. Seven of the donor females were killed at the time of the operation for histological study; the remaining seven died aged 21 to 34 days, presumably from the effects of the *scurfy* gene. In all operations, one host female was used for each donor female. Both of the host ovaries were removed, and each emptied capsule immediately received either a whole donor ovary, or, in some cases, a half donor ovary (the other ovary being used for histological study).

Offspring of Scurfy Females.—Of the 14 operations performed, 7 resulted in young shown by one means or another to have been definitely derived from the transplanted ovaries of *scurfy* females. (It is interesting to note that such a high percentage of success was obtained. This indicates that in those operations in which compatibility could not be made perfect, the choice of (101 × C3H)F_1 hosts was a good one.) Four of the host females produced no young, and three gave only two or three young each, none carrying the required markers.

Of course, any *scurfy* or *scurfy*-transmitting animals must come from the transplanted ovaries, even if the coat-color marking system was not by itself adequate to establish this. However, it is important to consider the ratio of *scurfy* to non-*scurfy* and of *scurfy*-transmitting to non-transmitting animals among those young whose origin from the transplanted ovaries of *scurfy* females could be established solely on the basis of markers other than *sf* itself. Table 1 is limited to these young.

The young omitted from Table 1 are a mixed group. Some, namely, 2 *scurfy* males and 8 *scurfy*-transmitting females (to be described below), must definitely

TABLE 1

OFFSPRING FROM OVARIES OF *Scurfy* FEMALES TRANSPLANTED TO NORMAL HOSTS (THESE OFFSPRING ARE IDENTIFIED BY COAT COLOR MARKERS AS NOT BEING FROM REGENERATED HOST OVARIAN TISSUE)

		Sons		Daughters	
Ovary Donor	Host Strain	*Scurfy*	*Normal*	Normal, Transmit *Scurfy*	Normal, Do Not Transmit *Scurfy*
D ♀ 1320	(101 × C3H)F_1	3	0	0	0
D ♀ 2570	(101 × C3H)F_1	1	0	3	0
D ♀ 5403	129	6	0	3	1
D ♀ 5542	(101 × C3H)F_1	1	0	2	5
Ov ♀ 15	(101 × C3H)F_1	7	0	8	4
D ♀ 6859	129	1	0	0*	0*
Total		19	0	16	10

* Two female offspring of this ovary have not yet been tested sufficiently to show whether or not they transmit *scurfy*.

have come from transplanted ovaries; others, namely, those from the 129-strain hosts, were identified by their markers as coming from regenerated host ovaries. The remaining 6 male and 11 female young could have come from either transplant or regenerate ovaries (although it seems highly likely, in the light of the results from the color-marked young, that the males are from the latter).

The striking feature of the results, apparent from Table 1, is that all sons of *scurfy* females are *scurfy*, while the daughters, all of whom are phenotypically wild-type, fall into two groups: those transmitting and those not transmitting *scurfy*.

The Genetic Nature of Scurfy Females.—Six possible explanations for the occurrence of *scurfy* females are considered below. One (hypothesis 1) proposes that the exceptional females are heterozygous; two (hypotheses 2 and 4) that they are homozygous; and three (hypotheses 3, 5, and 6) that they are hemizygous for the mutant *sf*. As will be shown, it was possible to discard hypotheses 1, 2, and 3 on the basis of the results presented in Table 1. But, until the findings described in the accompanying paper[1] became available, no decision was possible between the three remaining hypotheses, each of which involved seemingly unlikely assumptions.

(1) *Loss of recessiveness of sf:* It was conceivable that, possibly as a result of modifiers, *scurfy* occasionally acted as a dominant. Such a hypothesis was thought of mainly in connection with the possibility that *scurfy* was sex-limited rather than sex-linked (i.e., acting as a dominant in males but usually as a recessive in females). The fact that all 19 of the sons of ovaries from *scurfy* females were *scurfy* disproves the hypothesis of loss of recessiveness, and thus simultaneously removes the possibility that *scurfy* is sex-limited. Conclusion: (*a*) *sf* is on the *X*-chromosome; (*b*) *scurfy* females are not of genotype X^{sf}/X^{+}.

(2) *High rate of mutation of* $+^{sf}$ *to sf:* The frequent occurrence of new *sf* genes might, with certain qualifications, account for X^{sf}/X^{sf} females. However, the fact that the progeny of *scurfy* females contains daughters not transmitting *scurfy* disproves this hypothesis.

(3) *Sex-reversal:* According to this hypothesis, *scurfy* females were of genotype X^{sf}/Y, converted to a female phenotype through the action of other factors. Were this the case, however, half of the sons of ovaries from *scurfy* females should have been non-*scurfy*, and all of the daughters should have transmitted *scurfy*. Neither of these two consequences was realized in the actual findings. The hypothesis was,

therefore, disproved, unless it is assumed that the female phenotype of the XY traces to some Y-chromosome abnormality, e.g., deficiency of male-determining factors. This special condition, however, approaches hypothesis 6.

(4) *Non-disjunction of scurfy in the mother:* According to this hypothesis, *scurfy* females were $X^{sf}/X^{sf}/Y$, with the X's unattached. Females of this type would produce gametes predominantly of types X^{sf} and $X^{sf}Y$, and rarely of types $X^{sf}X^{sf}$ and Y. The rare gametes would give rise to two probably lethal types, as well as to *scurfy* daughters and normal sons. The fact that these latter two classes were not found did not, in itself, disprove the hypothesis, since the X^{sf}/X^{sf} and Y gametes might have been formed too rarely to be detected in a small sample. The predominantly formed X^{sf} and $X^{sf}Y$ gametes would produce *scurfy* sons (X^{sf}/Y); a type ($X^{sf}/Y/Y$) which may be presumed to die (see below); and two types of wild-type daughters, X^{+}/X^{sf} and $X^{+}/X^{sf}/Y$. The results indeed showed *scurfy* sons and two types of wild-type daughters, namely, those transmitting and those not transmitting *scurfy*. But the latter class presented a stumbling block: the only manner in which the non-transmitting females could be reconciled with this hypothesis was by assuming that they were of the type $X^{+}/X^{sf}/Y$, that their $X^{sf}/Y/Y$ sons died prenatally, and that, through some unknown affinity, the maternal Y-chromosome segregated together with the maternal X^{sf} chromosome instead of with the paternal X^{+} chromosome. This seemed unlikely. On the other hand, in favor of this hypothesis was the fact that one presumed X^{+}/X^{sf} daughter of an ovary from a *scurfy* female produced three more exceptional *scurfy* females (including Ov ♀ 15, see Table 1), as if a high non-disjunction line had been initiated.

(5) *Spontaneous deficiencies involving the $+^{sf}$ allele:* According to this hypothesis, *scurfy* females were $X^{Df(+^{sf})}/X^{sf}$, and the non-transmitting daughters of *scurfy* ovaries were thus $X^{Df(+^{sf})}/X^{+}$. This hypothesis explained all of the breeding results, if it was assumed that $X^{Df(+^{sf})}/Y$ males died prenatally, rather than being of *scurfy* phenotype. The only objection to this hypothesis was that it implied a rate of spontaneous deficiency several orders of magnitude greater than even the maximum rate for autosomes that could be calculated from our mutation-rate experiments[3] (see frequency of *scurfy* females, below).

(6) *A sex-determining mechanism by which X/O is female:* This hypothesis (suggested by W. J. Welshons), according to which *scurfy* females were X^{sf}/O and their non-transmitting daughters were X^{+}/O, was consistent with all of the results. However, if true, it means that the sex M determining mechanism in mice was different from that in *Drosophila*, where X/O is male. It also meant that the rate of loss of the paternal sex chromosome was high.

Thus, of the six hypotheses considered, the data from ovarian transplant offspring disproved three. Of the remaining three, each involved assumptions that were seemingly unlikely. However, the assumptions for hypothesis 6 now appear, from recent work,[1] to be justified. This hypothesis seems, therefore, to provide the most likely explanation of the results. It is hoped to make a final genetic test of it by mating daughters of transplanted ovaries from *scurfy* females to X^{Ta}/Y males to see if those which fail to transmit *sf* are X^{+}/O. In addition, cytological analysis can be made of these females and of new occurrences of *scurfy* females. In the meantime, *scurfy* females will be considered, in the remaining discussion, to be of genotype X^{sf}/O.

Viability and Fertility of X/O Females.—Data on the performance of the daughters of ovaries from *scurfy* females can now be examined from the point of view that these daughters are of the two types X^+/X^{sf} and X^+/O. The data are summarized in Table 2. The 24 daughters who transmitted *scurfy*, and are presumed to be X^+/X^{sf}, include the 16 shown in Table 1 and an additional 8 omitted from Table 1 for reasons, stated above, which do not, however, invalidate their being used in the present comparison. The 10 daughters who did not transmit *scurfy*, but are known from their other markers to have come from transplanted ovaries (Table 1), are presumed to be X^+/O. A total of 1,543 and 368 offspring from the two types, respectively, were observed.

TABLE 2

OFFSPRING OF DAUGHTERS OF TRANSPLANTED OVARIES FROM EXCEPTIONAL *Scurfy* FEMALES

	Offspring of 24 presumed X^+/X^{sf} ♀♀		Offspring of 10 presumed X^+/O ♀♀	
No. of litters per female	8.6		8.8	
Average litter size born*	7.8		4.3	
Average litter size weaned*	5.1		3.7	
Total no. of young born per female†	64.3		36.8	
	♂♂	♀♀	♂♂	♀♀
Scurfy, died young‡	219	1	...	...
Scurfy, weaned	123	2	...	...
Normal, died young‡	14	7	0	0
Normal, weaned	372	578	144	180
Unclassified, died young‡	110	113	23	20
Total	838	701	167	200

* Excludes a few litters born after females had reached the age of 1 year.
† Includes a few animals not classified for sex.
‡ Before 25 days of age.

X^+/X^{sf} and X^+/O females are expected to produce four classes of young each. Of these, two are the same in the two cases, i.e., each type of mother produces X^+/X^+ and X^+/Y. In addition, the X^+/X^{sf} mothers produce X^+/X^{sf} and X^{sf}/Y, while the X^+/O mothers produce X^+/O and Y/O. The Y/O type might be expected to be lethal, and the following considerations support this expectation. Thus, if Y/O is lethal, i.e., one-half of all males die, the proportion of males in the offspring of X/O females ought to be approximately two-thirds that in the offspring of X/X females. However, the unadjusted proportions cannot be compared because of evidence for loss of X/O daughters on one side of the comparison and evidence for reduced viability of *scurfy* sons on the other. Adjustments can be made (*a*) on the basis of the observed frequencies of X/X and X/O progeny in Table 1 of this paper and Table 2 of the accompanying paper[1] (the combined data indicating X/O to be only 60 per cent as viable as X/X); and (*b*) on the basis of normal male progeny from the X^+/X^{sf} females. When these adjustments are made, the proportion of males in the offspring of X^+/O females comes out to be 0.72 or 0.69 of the proportion in the offspring of X^+/X^{sf} females, depending, respectively, on whether the calculation includes or excludes animals not classified for *scurfy*. Thus, the results are in close agreement with the value of 0.67 expected if the Y/O class is lethal prenatally.

Thus, the lower number of young from X^+/O than from X^+/X^{sf} females can be at least largely accounted for by the total absence of the Y/O and the lower

viability of the X/O class in the progeny of the X^+/O females. In addition, certain features of the data indicate that, independent of the fate of the progeny, the X^+/O mothers are, in general, somewhat less successful than the X^+/X^{sf} mothers, in that all classes of young are slightly reduced in number. Thus, the average number of normal sons per litter from X^+/O is 1.64, as against 1.87 from X^+/X^{sf}; and the average number of classified daughters per litter from X^+/O would be 2.56 (following adjustment for inviability of X/O daughters, see above), as against 2.84 from X^+/X^{sf}.

Frequency of Primary Occurrence of X/O—Since, in a stock maintained by mating X^+/X^{sf} females with X^+/Y males, X/O females are detected only if the maternal X-chromosome carries *scurfy*, the frequency of occurrence of X/O must be based on the frequency of X^+/X^{sf}. The actual number of X^+/X^{sf} is not known, but since our stock-maintenance data indicate that X^+/X^{sf} and X^+/X^+ females in the stock are approximately equal in number, the frequency of occurrence of X/O may be computed from the ratio of *scurfy* females to half the total number of females in the stock and turns out to be 0.9 per cent. It should be borne in mind that this is probably an underestimate, since *scurfy* females are quite inviable and some undoubtedly died before detection. In view of this, it is not surprising that the incidence of X/O computed for the *scurfy* stock is slightly lower than the percentage computed from our data on other stocks.[1]

The data are insufficient to show definitively whether the occurrence of *scurfy* females is random. The 29 primary occurrences of X^{sf}/O were distributed among 25 sibships: 22 contained a single X^{sf}/O female each, two sibships contained two, and one contained three. Rough calculations indicate that the frequency of sibships with 2 or 3 cases of *scurfy* females is higher than expected on the basis of random distribution among sibships of average size. This indication of nonrandomness is strengthened by the observation that 2 of the 8 exceptions listed in the accompanying paper[1] were littermates. However, an accurate test of statistical significance, which would encounter a number of complications, has not yet been attempted. Although it may have no importance, it should perhaps be mentioned that the sibship containing 3 *scurfy* females consisted of second generation descendants of a *scurfy* female.

It is interesting to speculate at what stage the loss of the paternal sex-chromosome occurs. It may occur in spermatogenesis or, possibly, by non-disjunction in the first cleavage division (with the other aneuploid blastomere dying).

Summary.—A sex-linked recessive gene, *scurfy*, that kills male mice before they reproduce, occasionally shows exceptional inheritance in producing the *scurfy* phenotype in females. The genetics of such females, which also die before reproductive age, was analyzed by transplanting their ovaries and obtaining offspring from them. Several possible explanations for these females could be ruled out by the results of the progeny tests. Of the remaining possibilities, the one that now appears most likely is that the *scurfy* females have the sex-chromosome constitution X^{sf}/O. That X/O is female in the mouse is proved by independent data in the accompanying paper.[1] In the *scurfy* stock, the frequency of primary occurrence of X/O is 0.9 per cent, or possibly greater. The X^+/O daughters of X^{sf}/O ovaries are probably of somewhat reduced viability, but those that survive to weaning age are fertile. Their Y/O progeny probably die prenatally.†

* Operated by Union Carbide Nuclear Company for the United States Atomic Energy Commission.

† The formal reporting of the gene *scurfy*, apparently the first sex-linked gene to be discovered in the mouse, was, of course, long overdue, and some explanation for the delay seems in order. Although *scurfy* behaved, in general, like a sex-linked gene, the early, and not too infrequent, occurrence of *scurfy* females, here reported, raised the possibility that we were dealing, instead, with a sex-limited gene which was occasionally expressed in females. By 1951, the ovarian transplantation results had, as shown in this paper, excluded this possibility. Proof of sex-linkage was considered adequate at this time, when tabulation of the offspring from transplanted *scurfy* ovaries showed all of 13 sons to be *scurfy*. At this same time, there were 7 adequately tested daughters, all of which transmitted *scurfy* to half of their male offspring. This led us to the conclusion that the exceptional *scurfy* females might be the result of an unexpectedly high frequency of non-disjunction in their mothers. The stock was turned over to an assistant to collect about twice as much material. Owing to the pressure of other work we did not look at the augmented data until some time later, when the keen interest of Dr. Curt Stern in our apparently high frequency of non-disjunction led us to tabulate the complete data. We then found out, for the first time, that some of the more recently obtained daughters of *scurfy* ovaries were *non*-transmitters of *scurfy*. It was our bad luck that of the first 7 adequately tested daughters *all* were transmitters of *scurfy*, and that we consequently had no inkling that the later data would contain an exciting new problem. When this problem turned up, hypotheses 4, 5, and 6, outlined in this paper, were proposed as possible explanations. Some time before this, an exceptional female had occurred in another stock maintained by one of us (LBR), in a cross of $X^{+Ta}/X^{26K+} \times X^{++}/Y$, and it was decided that exceptional sex-linked inheritance might be more easily analyzed in *Tabby* crosses, where ovarian transplantation is not necessary. The genetic and cytological results obtained with these crosses are reported in the accompanying paper.[1] They indicate that X/O is female, a finding which explains the exceptional females not only in the *Ta* crosses, but also in the old *scurfy* data. Thus, the remaining problem in the *scurfy* results has apparently been resolved, and the data are now at last presented.

[1] Welshons, W. J., and L. B. Russell, these PROCEEDINGS, **45**, 560 (1959).

[2] Russell, W. L., and J. G. Hurst, these PROCEEDINGS, **31**, 267–273 (1945).

[3] Russell, W. L., L. B. Russell, and E. M. Kelly, *Science*, **128**, 1546–1550 (1958).

THE Y-CHROMOSOME AS THE BEARER OF MALE DETERMINING FACTORS IN THE MOUSE

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Introduction.—Russell, Russell, and Gower, in the accompanying paper,[1] report the occasional occurrences, over the course of several years, of an unexpected class of female progeny in matings of normal males with females heterozygous for the sex-linked mutation *scurfy*. These rare, unexpected, females phenotypically resemble the hemizygous males. Since the affected females die before reproducing, genetic analysis had to be attempted by means of ovarian transplantation. This was successful in several cases and the results, described in the companion paper,[1] ruled out a number of possible explanations for the exceptional *scurfy* females. Without further work, however, no decision was possible between the remaining hypotheses. The experiments to be described here have led to an unequivocal explanation of unexpected *X*-linked inheritance.